FIGURE 1

p1CLIP$_S$/HPV16$_{C+50-68+GUU}$
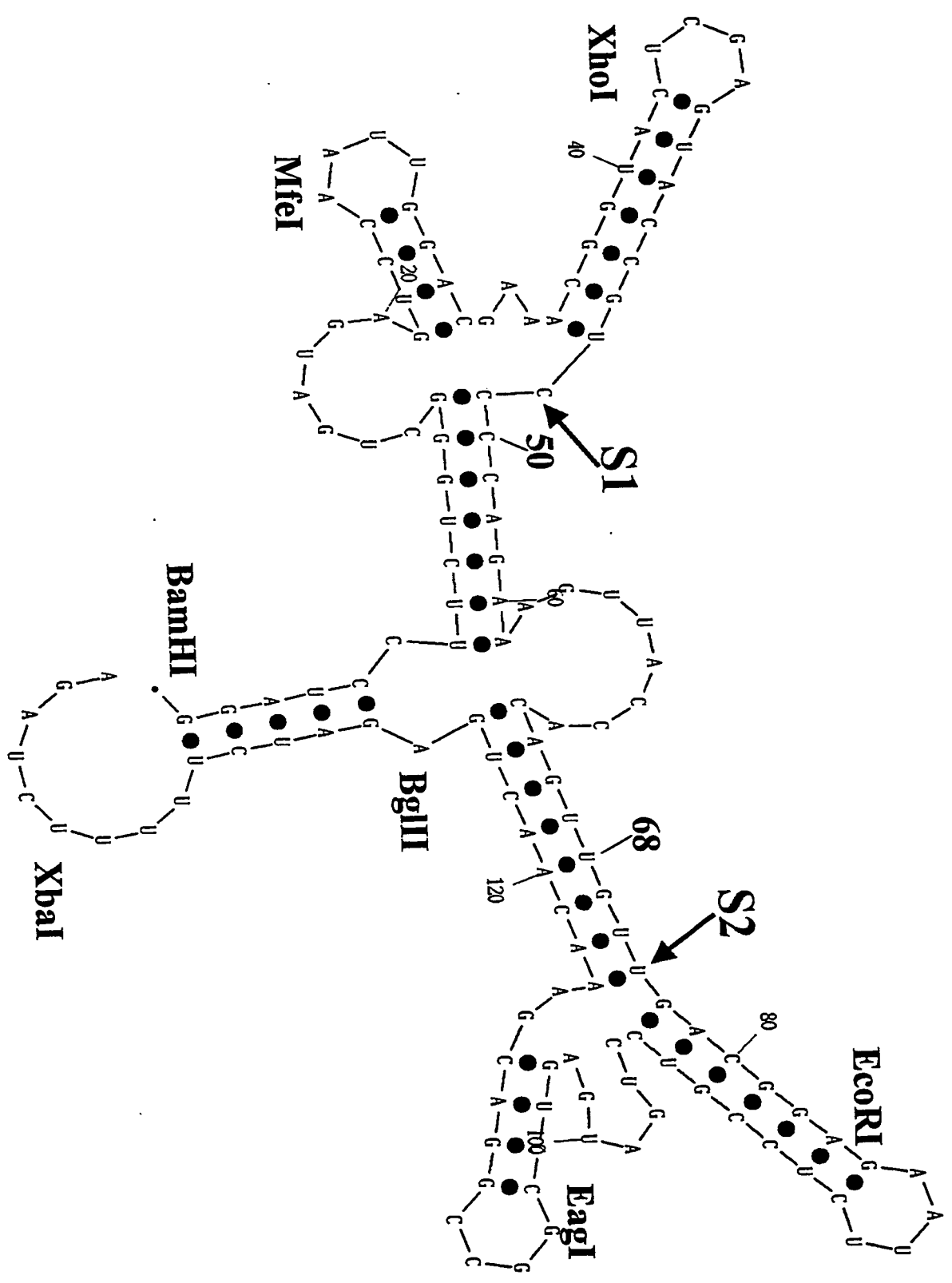
FIGURE 2

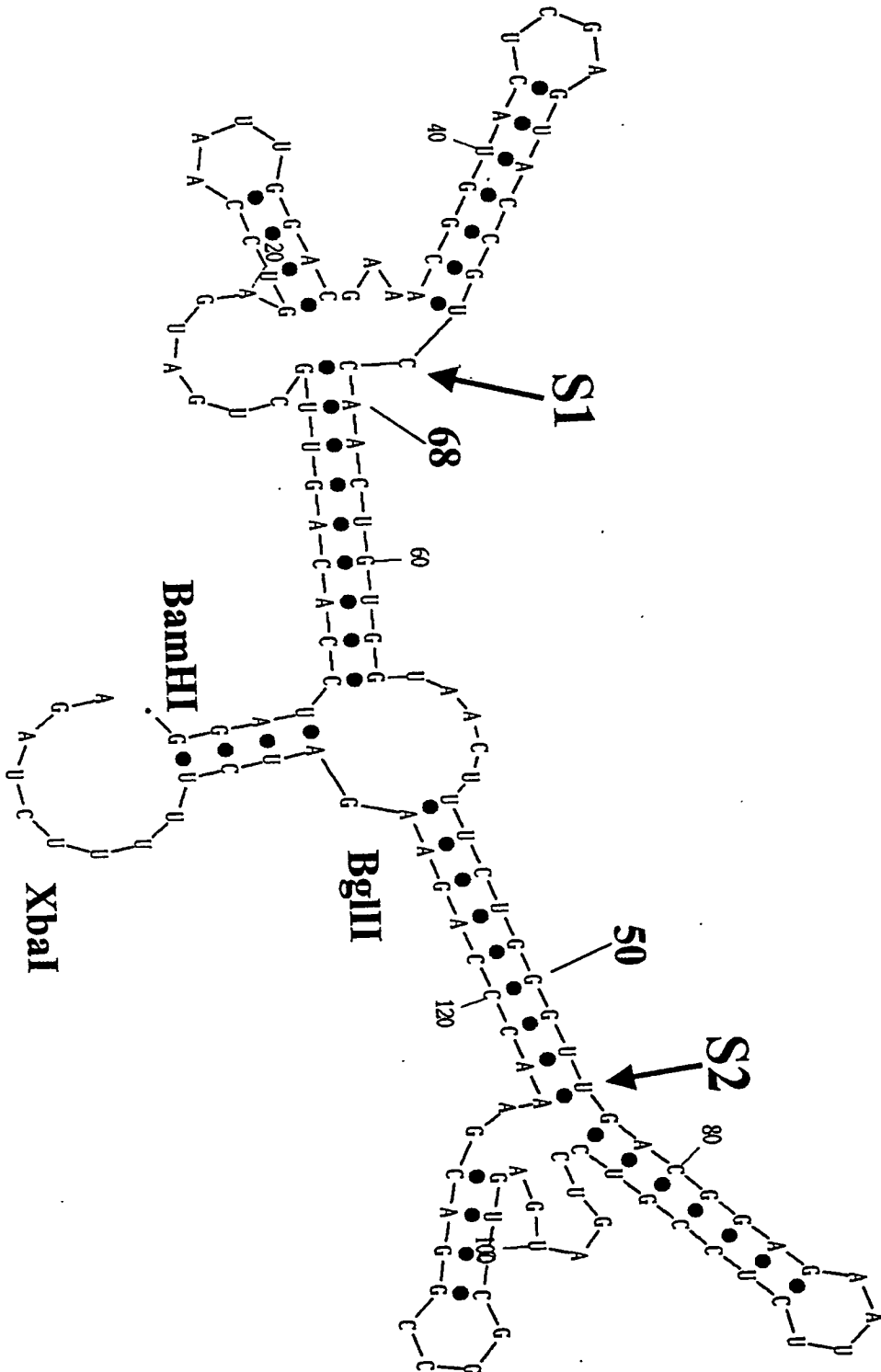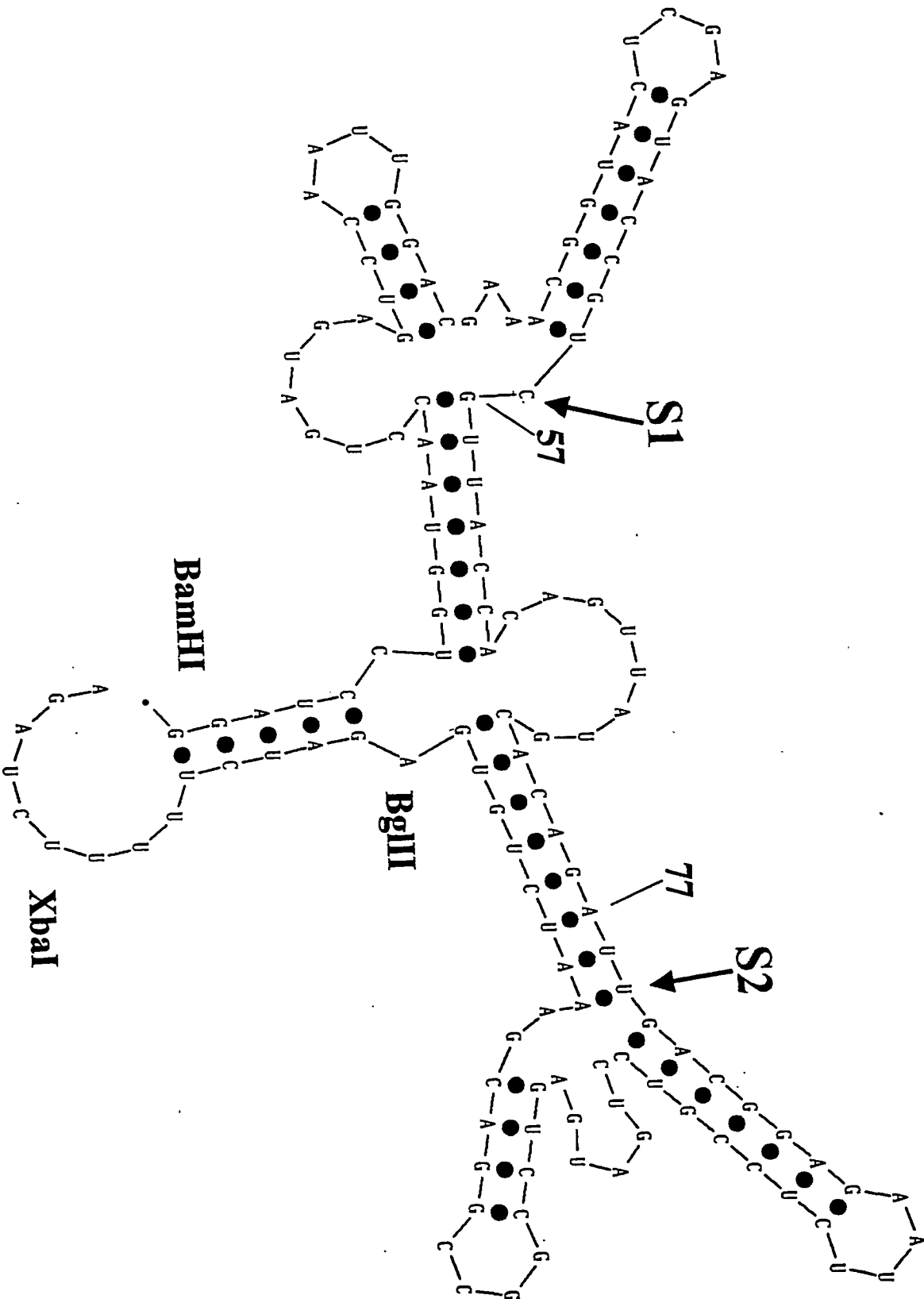pICLIP$_{AS}$/HPV16$_{C+68-50+GUU}$

FIGURE 3

p1CLIP$_S$/HPV16$_{57-77+UU}$
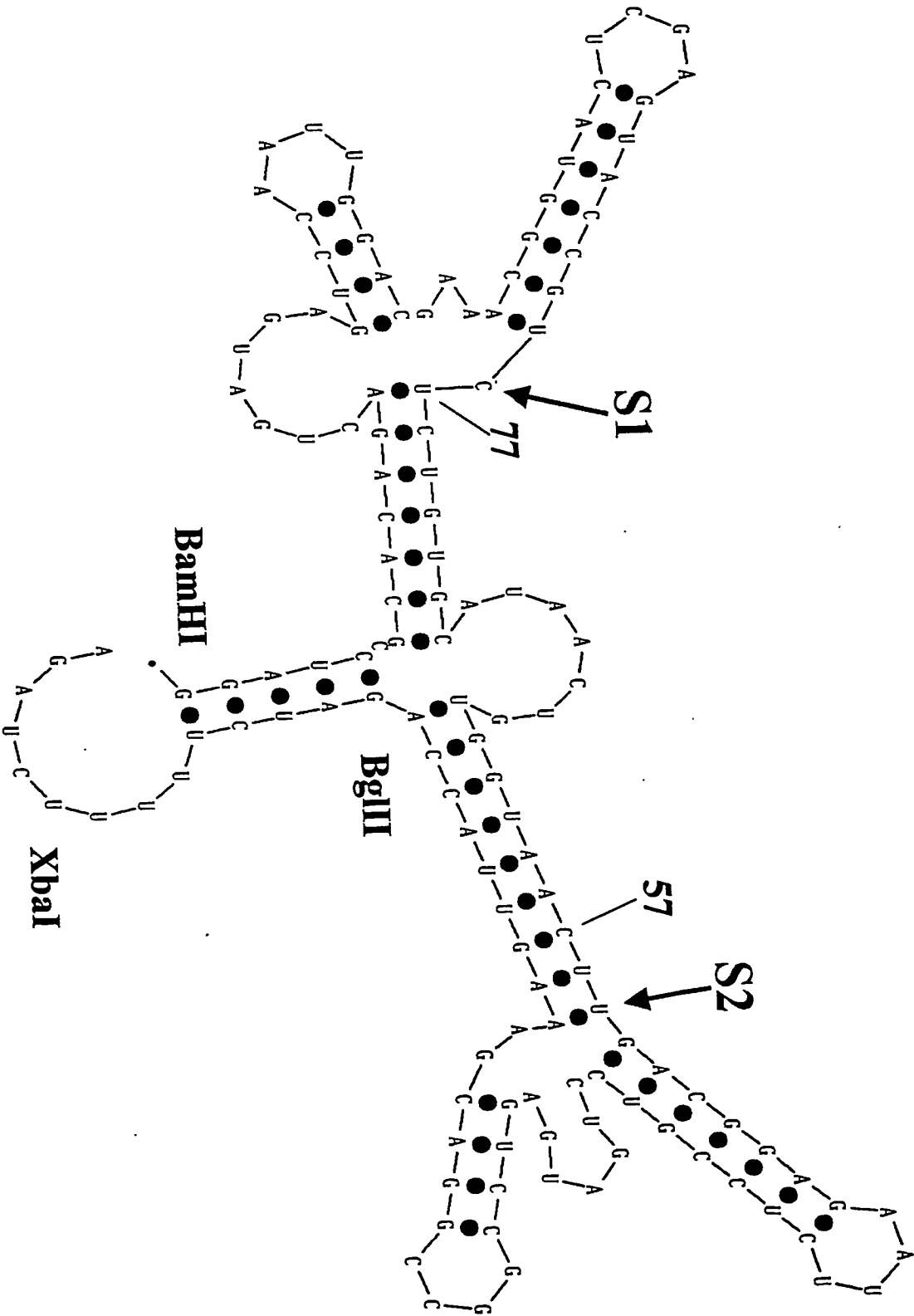
FIGURE 4

p1CLIP$_{AS}$/HPV16$_{77-57+UU}$

FIGURE 5

p2CLIP$_S$/HPV16$_{C+50-68+GUU}$
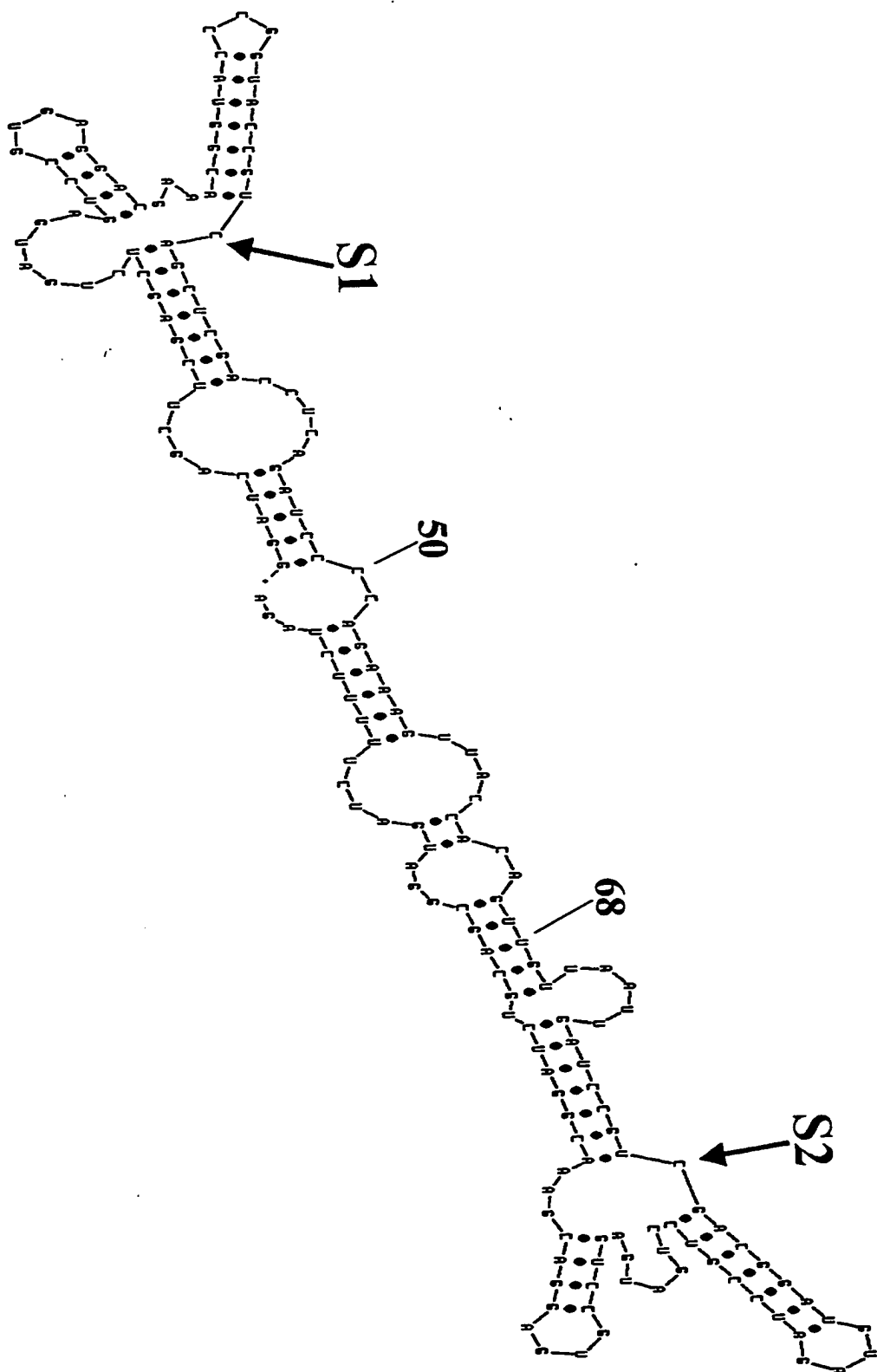
FIGURE 6

p2CLIP$_{AS}$/HPV16$_{C+68-50+GUU}$
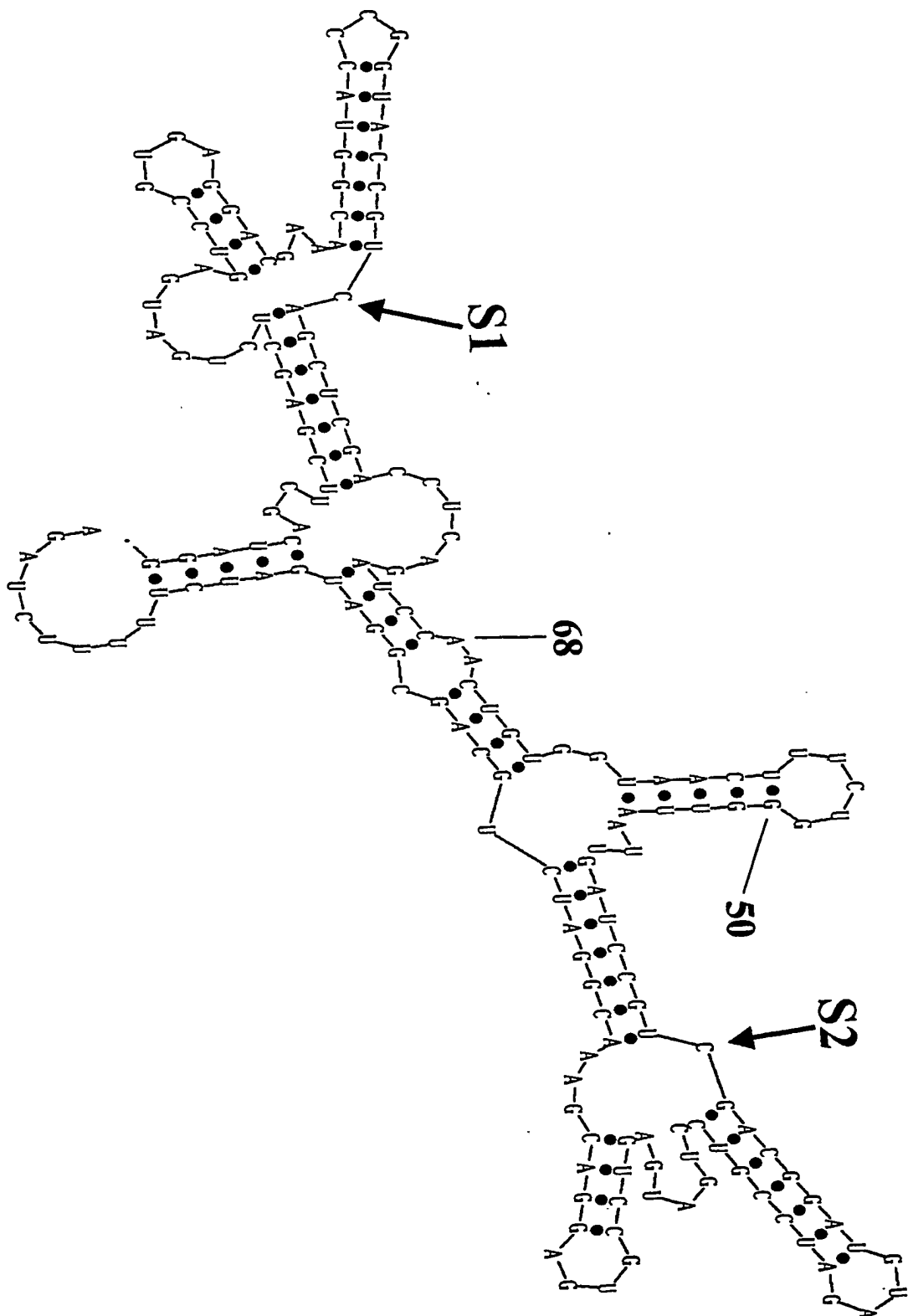
FIGURE 7

p1CHOP$_S$/HPV16$_{C+50-68+GUU}$
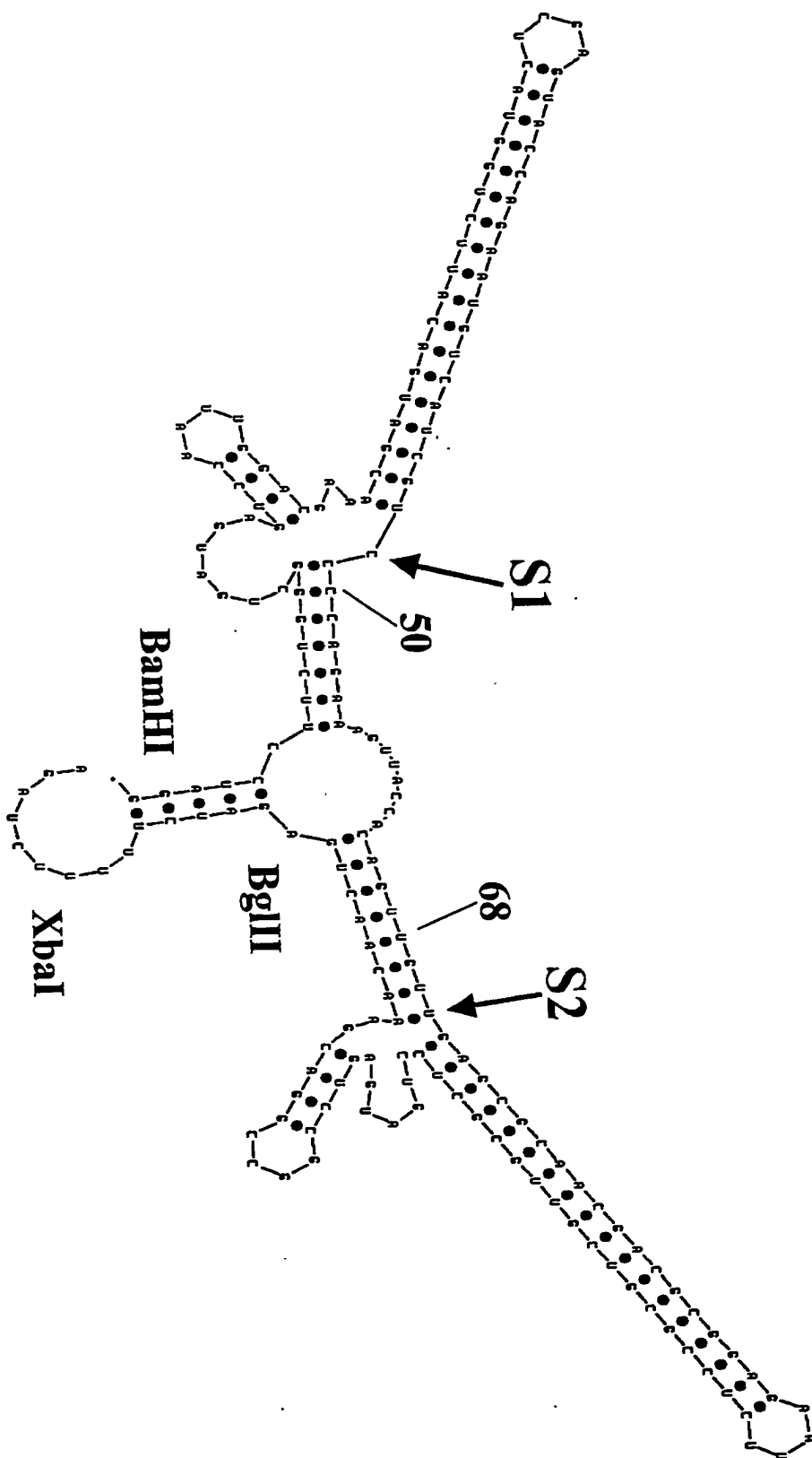
FIGURE 8

p1CHOP$_{AS}$/HPV16$_{C+68-50+GUU}$
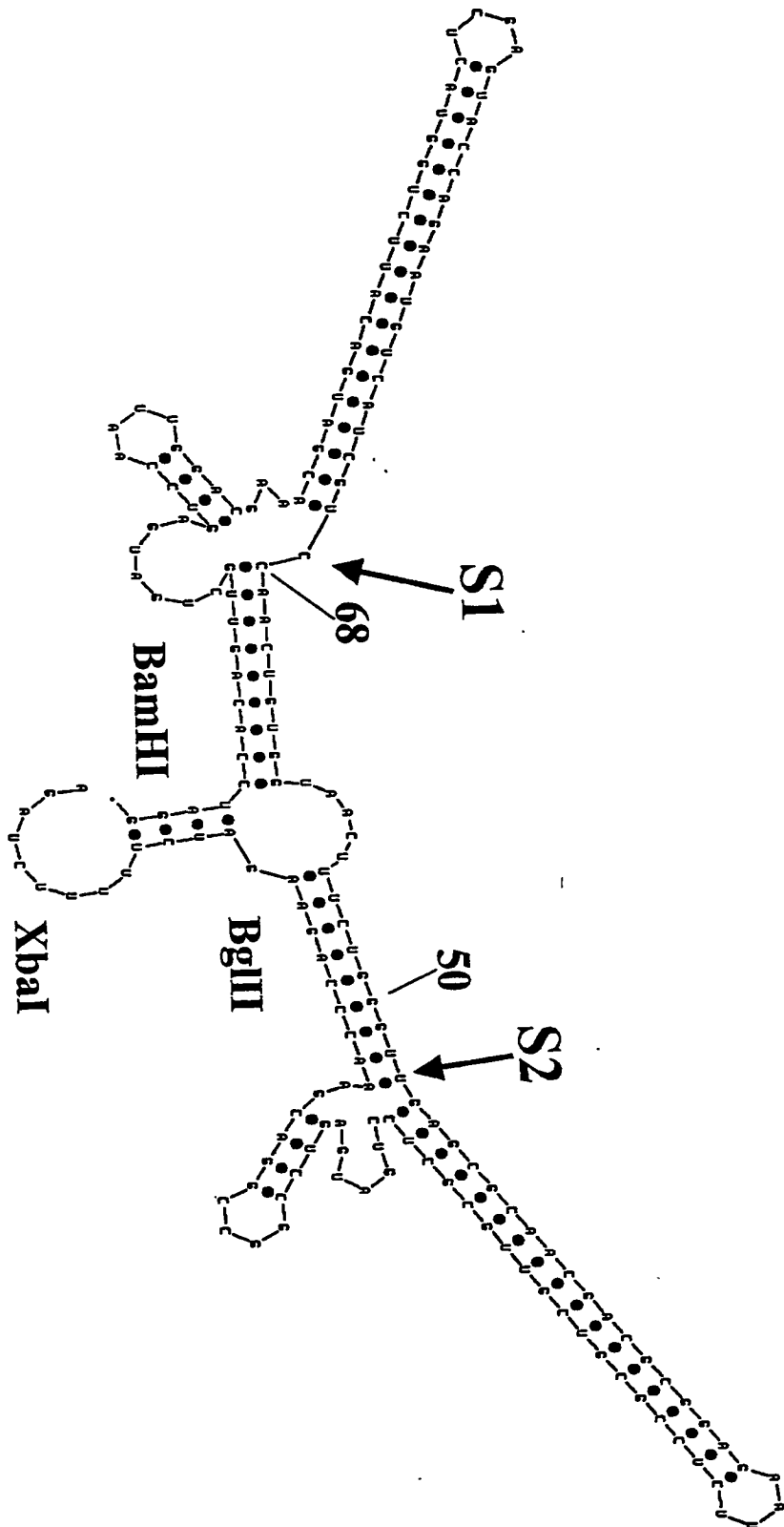
FIGURE 9

p2CHOP$_S$/HPV16$_{C+50-68+GUU}$

S1

S2

50

68
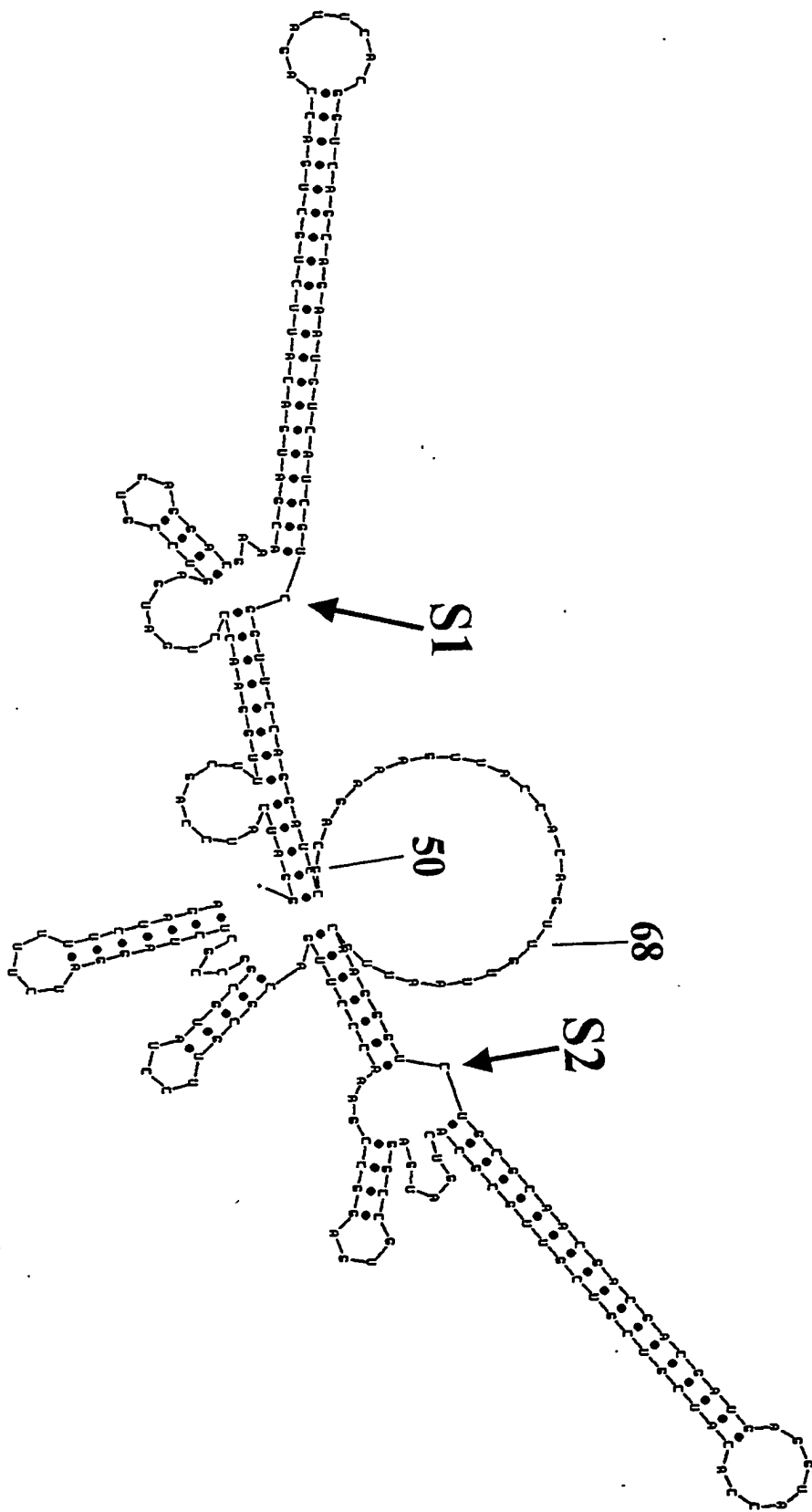
FIGURE 10

p2CHOP$_{AS}$/HPV16$_{C+68-50+GUU}$
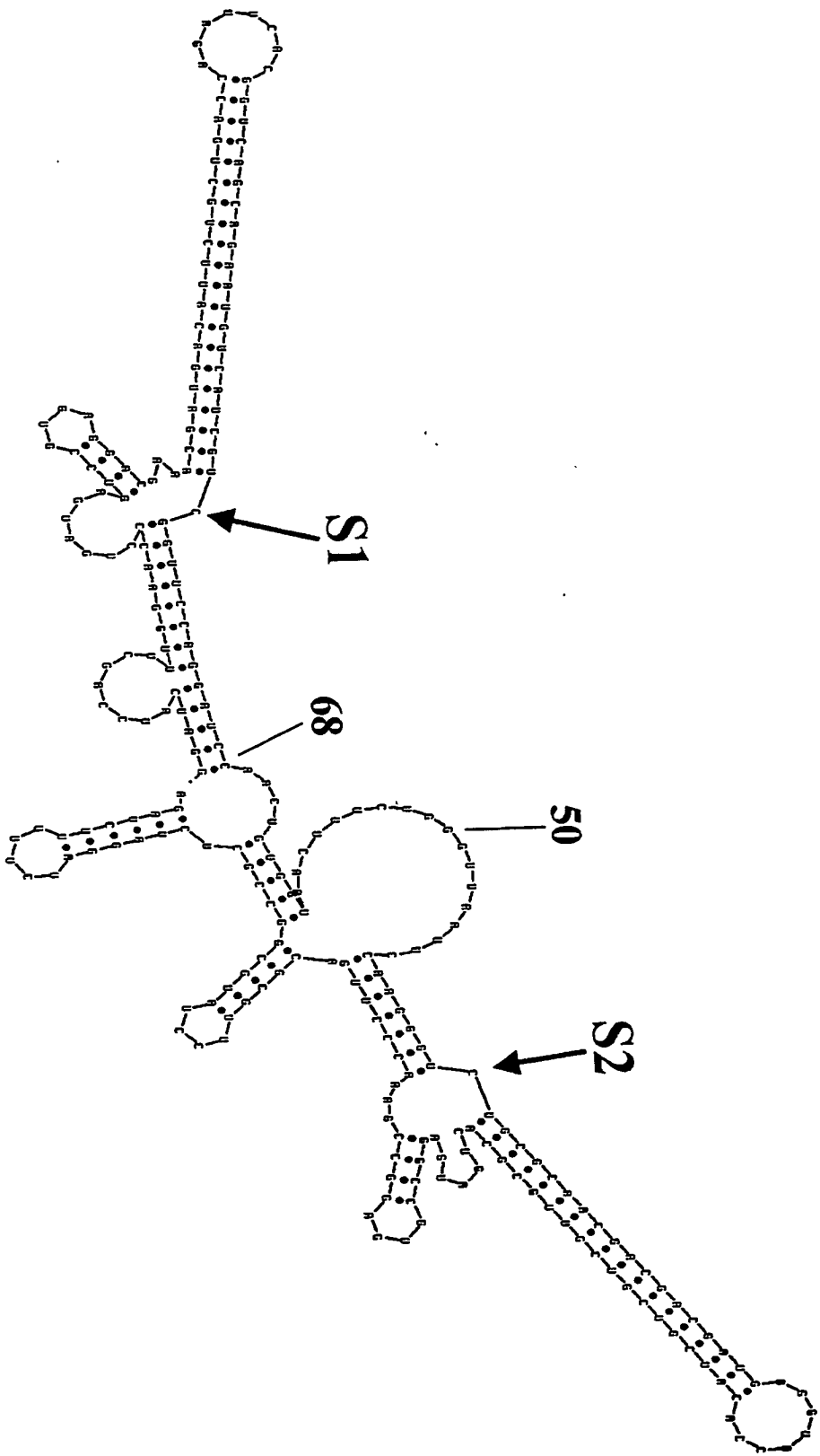
FIGURE 11

A. pSIR (Small Interfering RNA) Cassette

5'

S1

p(1/2)C(LI/HO)P (S/AS)

S2

p(1/2)C(LI/HO)P (S/AS)

S3

S4

3'
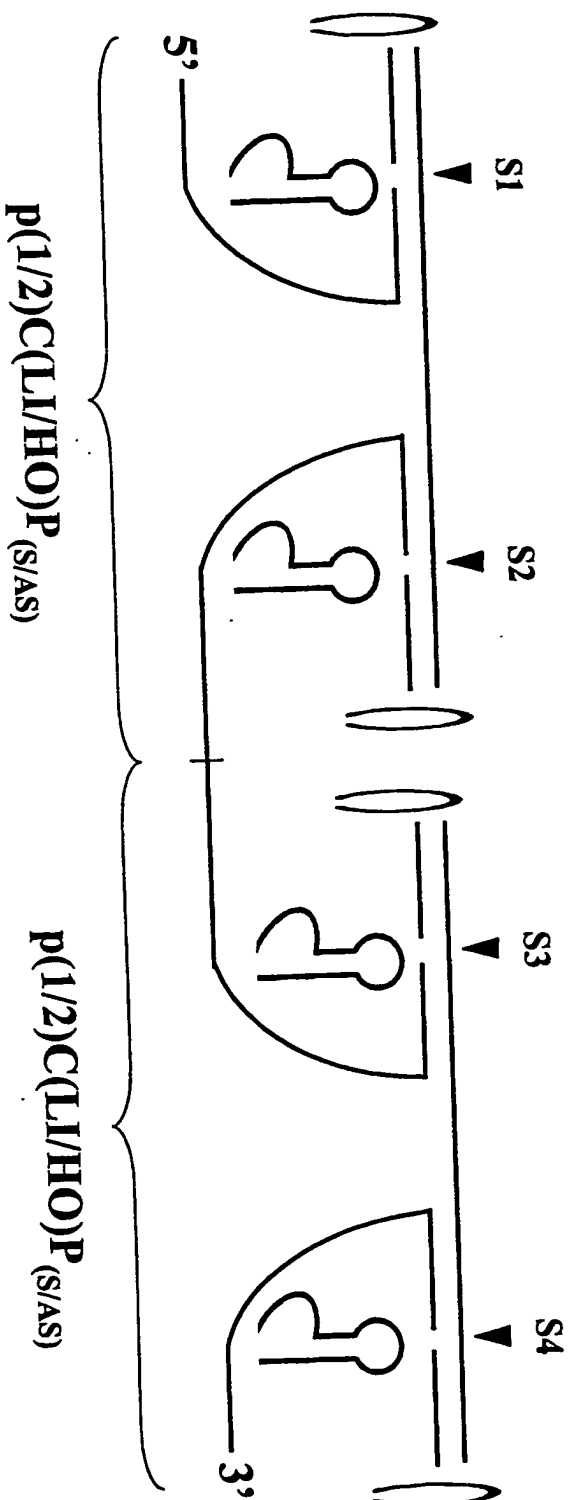
B. Expression Construct of pSIR Cassette

Tissue Specific Promoter

pSIR Cassettte

Hind III

BamHI

BglII TTT XbaI

VARIABLE

257 nt

UUUUU
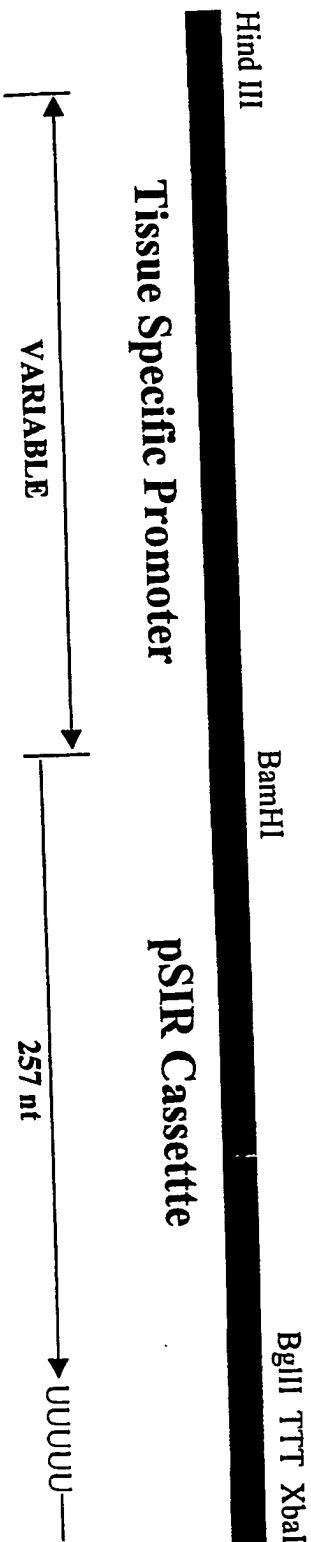
FIGURE 12

Self-Liberated Small Interfering (SI) RNA from pSIR Cassette

pSIR/HPV16si$_{C+50-68+GUU}$

```
S1-S2:    5'-C-CCAGAAAGUUACCACAGUU-GUU-3'    HPV16$_{50-68}$
S4-S3:    3'-UUG-GGUCUUUCAAUGGUGUCAA-C-5'    HPV16$_{68-50}$
```

pSIR/HPV16si$_{57-77+UU}$

```
S1-S2:    5'-GUUACCACAGUUAUGCACAGAUU-3'    HPV16$_{57-77}$
S4-S3:    3'-UUCAAUGGUGUCAAUACGUGUCU-5'    HPV16$_{77-57}$
```

pSIR/HBV16si$_{862-882+UU}$

```
S1-S2:    5'-UUUGGGAGCUACUGUGGAGUUAUU-3'    HBV$_{862-882}$
S4-S3:    3'-UUAAACCUCGAUGACACCUCAAU-5'    HBV$_{882-862}$
```

FIGURE 13

Self-Liberated Small Interfering (SI) RNA from pSIR Cassette

5' Sequence of p2CLIP: 5'-AGCUCGACCUCAGAUC (Bg1 II/Bam HI)-3'

3' Sequence of p2CLIP: 5'-(Mfe I/Eco RI) AAUUGAUCCGUC-3'

5' Sequence of p2CHOP: 5'-GGUUCCAGGAUC (Bg1 II/Bam HI)-3'

3' Sequence of p2CHOP: 5'-(Mfe I/Eco RI) AAUUCCAAGGGUC-3'

p2CLIP or p2CHOP/HPV16$_{C+50-68+GUU}$

5'-GAUCCCCAGAAAGUUACCACAGUUGUUAAUU-3'   HPV16$_{50-68}$

3'-UUAGGGGUCUUUCAAUGGUGUCAACAACAUG-5'   HPV16$_{68-50}$

p2CLIP or p2CHOP/HPV16$_{57-77+UU}$

5'-GAUCGUUACCACAGUUACGAGAUUAAUU-3'   HPV16$_{57-77}$

3'-UUAACAAUGGUGUCAAUACGUGUCUAACAUG-5'   HPV16$_{77-57}$

p2CLIP or p2CHOP/HBV$_{862-882+UU}$

5'-GAUCUUUGGGAGCUACUGUGGAGUUAUUAAUU-3'   HBV$_{862-882}$

3'-UUAAAACCUCGAUGACACCUCAAUAACAUG-5   HBV$_{882-862}$

FIGURE 14

p2CLIP$_{HR}$/HPV16$_{47-68}$
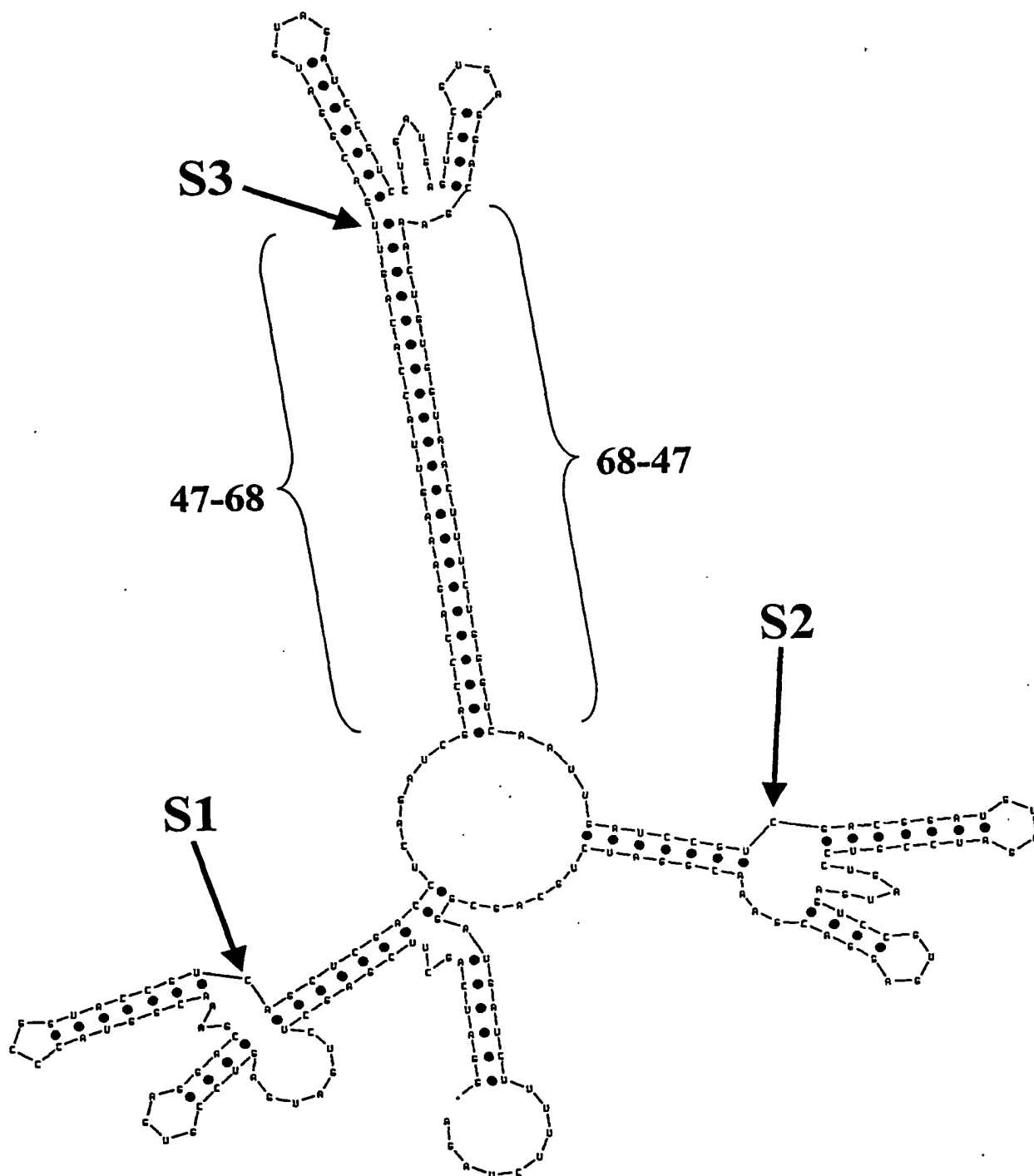
FIGURE 15

p2CHOP$_{HR}$/HPV16$_{47-68}$

S1

S3

47-68

68-47

S2
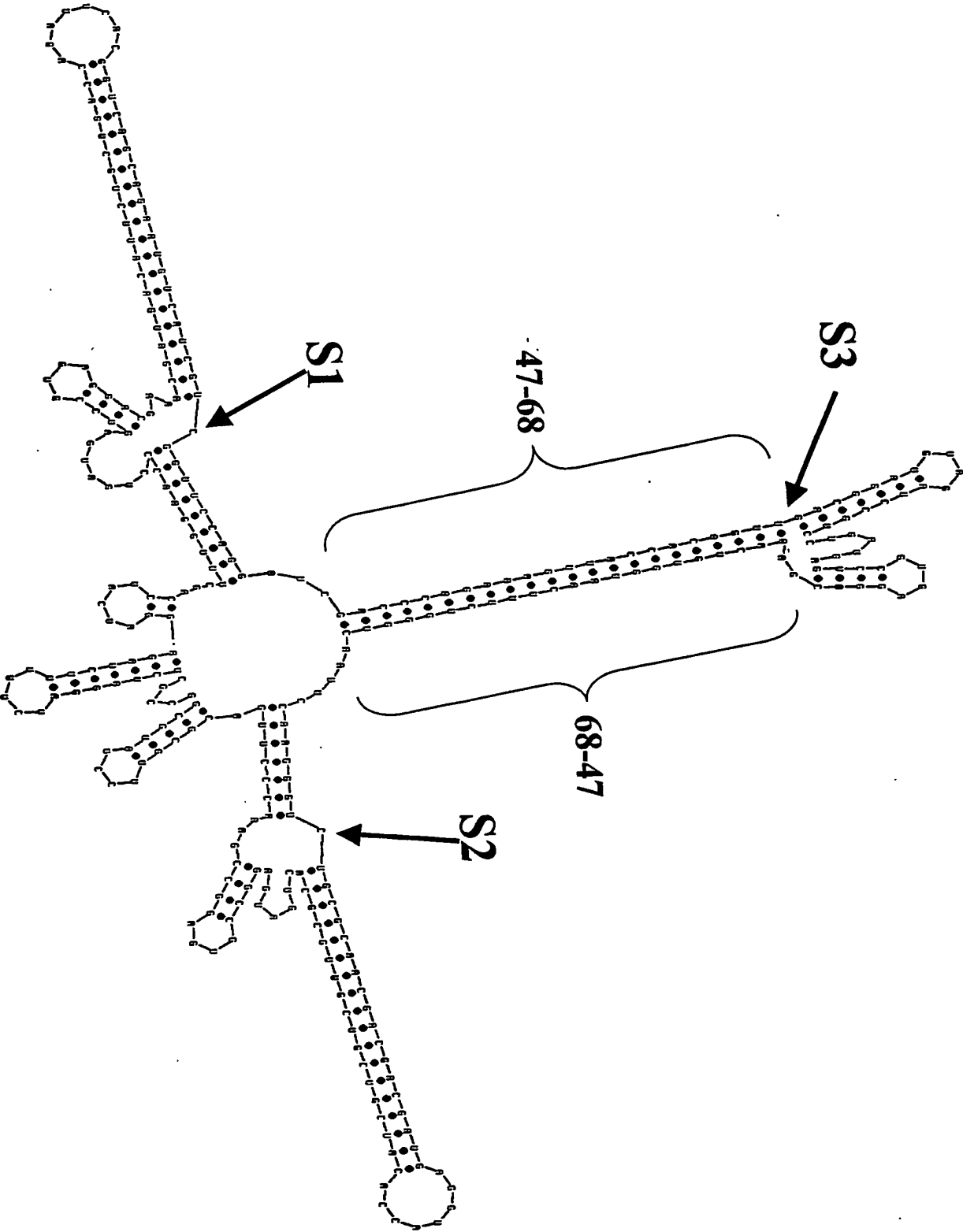
FIGURE 16

HR Inserts for Cassettes
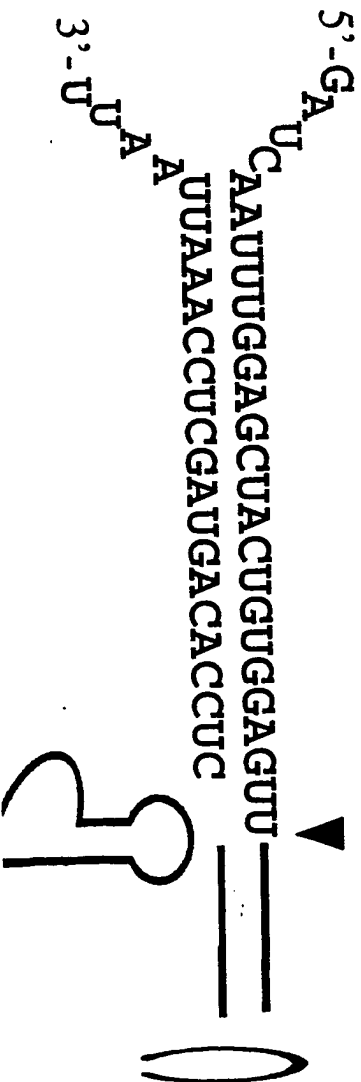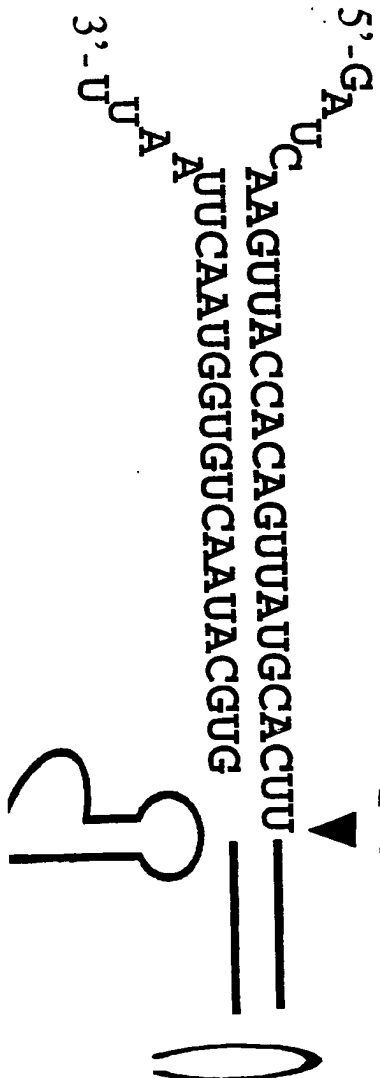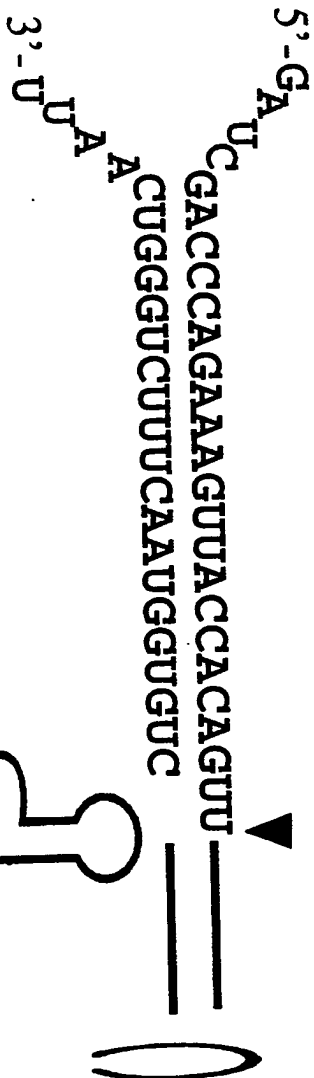
FIGURE 17

Hairpin with a Cis-Acting Rz (HR) in pSNIP Cassette
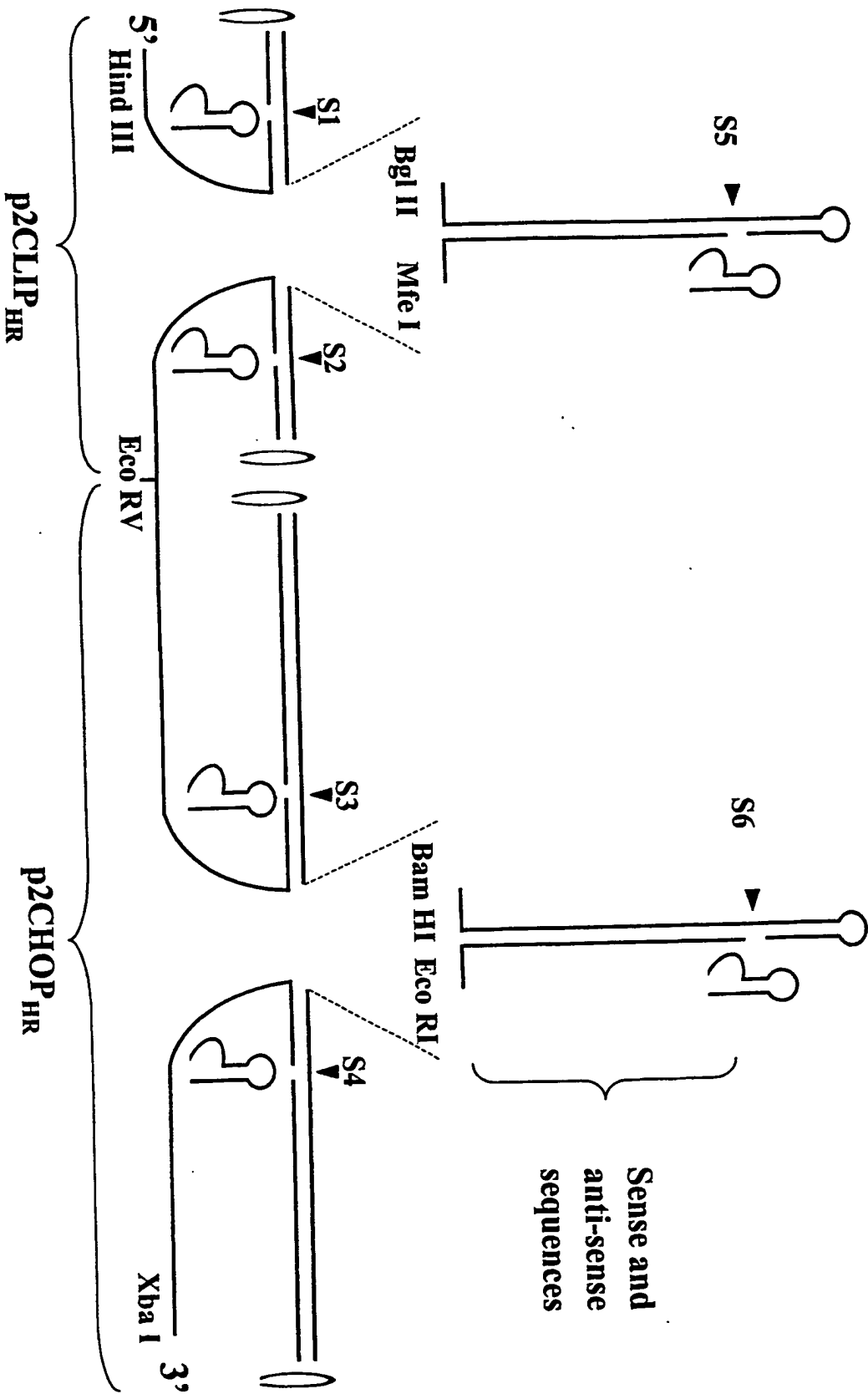
FIGURE 18

$$p2CLIP_{HP}/HPV16_{C+50-68+GUU}$$
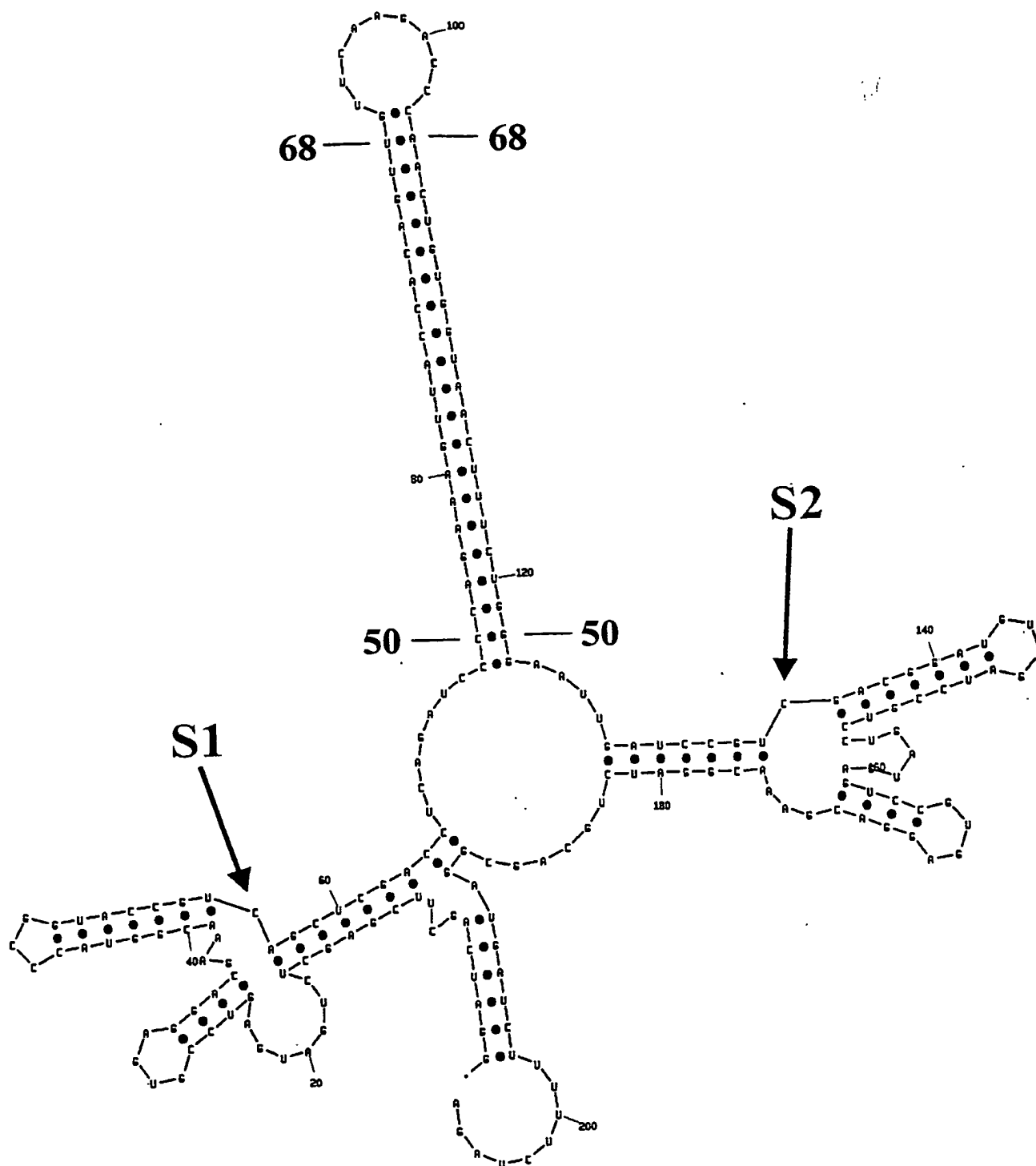
FIGURE 19

Self-Liberated Hair-Pin Loop (HP) from pCLIP/pCHOP Cassettes

HPV16$_{C+50-68+GUU}$

```
5'-CCCAGAAAGUUACCACAGUUG  U  U  C  A
3'-UUGGGUCUUUCAAUGGUGUCAAC  C  C  A  G
```

HPV16$_{57-77+UU}$

```
5'-GUUACCACAGUUAUGCACAGA  U  U  C  A
3'-UUCAAUGGUGUCAAUACGUGUCU  C  C  A  G  A  A
```

HBV16$_{862-882+UU}$

```
5'-TTTGGAGCTACTGTGGGAGTTA  U  U  C  A
3'-UUAAACCTCGATGACACCCTCAAT  C  C  A  G  A  A
```

FIGURE 20

FIGURE 21

A. Library Construction

5'-CCGAAGCTTAATACGAC-3'
5'-CCGAAGCTTAATACGACTCACTATAGGGATCGTCGGTTCCAGATC-3'

      3'-CTAGCAGCCAAGGTCTAGATTTTT-n21-AAAAATCTAGAGTTCCCAGACGC-5'

(a) PCR

T7 Promoter   BglII   BglII
5'-CCGAAGCTTAATACGACTCACTATAGGGATCGTCGGTTCCAGATCTAAAAA-N21-TTTTAGATCTCAAGGGTCTGCG-3'
3'-GGCTTCGAATTATGCTGAGTGATATCCCTAGCAGCCAAGGTCTAGATTTTT-n21-AAAAATCTAGAGTTCCCAGACGC-5'

(b) Transcription

5'-GGGAUCGUCGGUUCCAGAUCUAAAAA-N21-UUUUUAGAUCUCAAGGGUCUGCG-3'

B. Enrichment

5'-GGGAUCGUCGGUUCCAGAUCUAAAAA-E21-UUUUUAGAUCUCAAGGGUCUGCG-3'

Target RNA
Library RNA

+

(a) Annealing

RNA/RNA Complex

(b) RT-PCR

(c) Transcription

5'---AGATCTAAAAA-E21-TTTTAGATCT---3'
3'---TCTAGATTTTT-e21-AAAAATCTAGA---5'

C. BglII Digestion

5'-GATCTAAAAA-E21-TTTTTA-3'
3'-ATTTTT-e21-AAAAATCTAG-5'

FIGURE 22

Down-Regulation of HPV16 mRNA by siRNA Constructs in Cells

Figure 23

FIGURE 24

A. Library Construction

5'-CCGAAGCTTAATACGAC-3'
5'-CCGAAGCTTAATACGACTCACTATAGGGATCGTCGGTTCCAGATC-3'

(a) PCR

```
         T7 Promoter                              BglII              XbaI
5'-CCGAAGCTTAATACGACTCACTATAGGGATCGTCGGTTCCAGATCTAAAAA-N21-TTCTAGAACAGGGTCTGC-3'
3'-GGCTTCGAATTATGCTGAGTGATATCCCTAGCAGCCAAGGTCTAGAGATTTTT-n21-AAGATCTTGTCCCAGACG-5'
```

3'-CTAGCAGCCAAGGTCTAGAGATTTTT-n21-AAGATCTTGTCCCAGACG-5'

(b) Transcription

5'-GGGAUCGUCGGUUCCAGAUCUAAAAA-N21-UUCUAGAACAGGGUCUGC-3'

B. Enrichment

5'-GGGAUCGUCGGUUCCAGAUCUAAAAA-E21-UUCUAGAACAGGGUCUGC-3'

(a) Annealing

Target RNA
Library RNA

RNA/RNA Complex

(d) Transcription

(b) RT

(c) PCR

5'---TAAAAA-E21-TT---3'
3'---ATTTTT-e21-AA---5'

3'-CCCTAGCAGCCTTGGTCTAGAATTTTT-e21-AAGATCTTGTCCCAGACG-5'

FIGURE 25

```
5'-GGGATCGTCGGTTCCAGATC-3'
3'-CCCTAGCAGCCAAGGTCTAGATTTTT-e21-AAGATCTTGTCCAGACG-5'  cDNA from Figure 24
   BglII                                         XbaI
            3'-AAGATCTTGTCCAGACGTTCAAGTTCAAG-5'
               3'-TTCAAGTTCAAGTTCAAGCGTCTGGGACAAGATCTT-5'
                                                   XbaI
```

A. PCR   **(a) Addition of Hairpin**

```
5'-GGGATCGTCGGTTCCAGATCTAAAAA-E21-TTCTAGAACAGGGGTCTGCAAGTTCAAGTTCGCAGACCCTGTTCTAGAA-3'
   BglII                                                                        XbaI
```

(b) Cis-Extension

```
5'-GGGATCGTCGGTTCCAGATCTAAAAA-E21-TTCTAGAACAGGGGTCTGC A
                                                        AGTTCA  A
                                                     C          G
3'-CCCTAGCAGCCAAGGTCTAGATTTTT-e21-AAGAUCUGUCCCAGACG  T      T
   BglII                                  XbaI        TTGAAC
```

(c) Full-Length Amplification

```
5'-GGGATCGTCGGTTCCAGATCTAAAAA-E21-TTCTAGA--------TCTAGAA-e21-TTTTTAGATCTGGAACCGACGATCCC-3'
3'-CCCTAGCAGCCAAGGTCTAGATTTTT-e21-AAGAUCU--------AGATCTT-E21-AAAAATCTAGACCTTGGCTGCTAGGG-5'
   BglII                           XbaI           XbaI                                BglII
```

B. Editing   **(a) BglII/Cloning**

```
5'-GATCTAAAAA-E21-TTCTAGAACAGGGGTCTGCAAGTTCAAGTTCGCGAACCCTGTTCTAGAA-e21-TTTTTA-3'
3'-ATTTTT-e21-AAGATCTTGTCCAGACGTTCAAGTTCAAGCGTCTGGGACAAGATCTT-E21-AAAAATCTAG-5'
   BglII                          XbaI            XbaI              BglII
```

(b) XbaI/Re-ligation

```
5'-GATCTAAAAA-E21-TTCTAGAA-e21-TTTTTA-3'
3'-ATTTTT-e21-AAGATCTT-E21-AAAAATCTAG-5'
   BglII          XbaI       BglII
```

FIGURE 26

A. Construction of Expression Cassette

eGFP   Target   U6 Promoter   H1 Promoter

BamHI

GGATCC
CCTAGG

→ Inserts →

GGATCTAAAAAE21TTCTAGAAe21TTTTTAGATCC
CCTAGATTTTe21AAGATCTTE21AAAAAATCTAGG

B. Screening in Flp-In 293 Cells

(a) Transcription

5'-E21UU-3'
3'-UUe21-5'

(b)

DICER

5'-GGAUCUAAAAAE21UU ^{C} U
3'-UUUUUe21AA $_G$ A

RISC

(c)

C. PCR Sequence

5'-GGATCTAAAAA-X21-TTCTAGAA-Y21-TTTTTAGATCC-3'